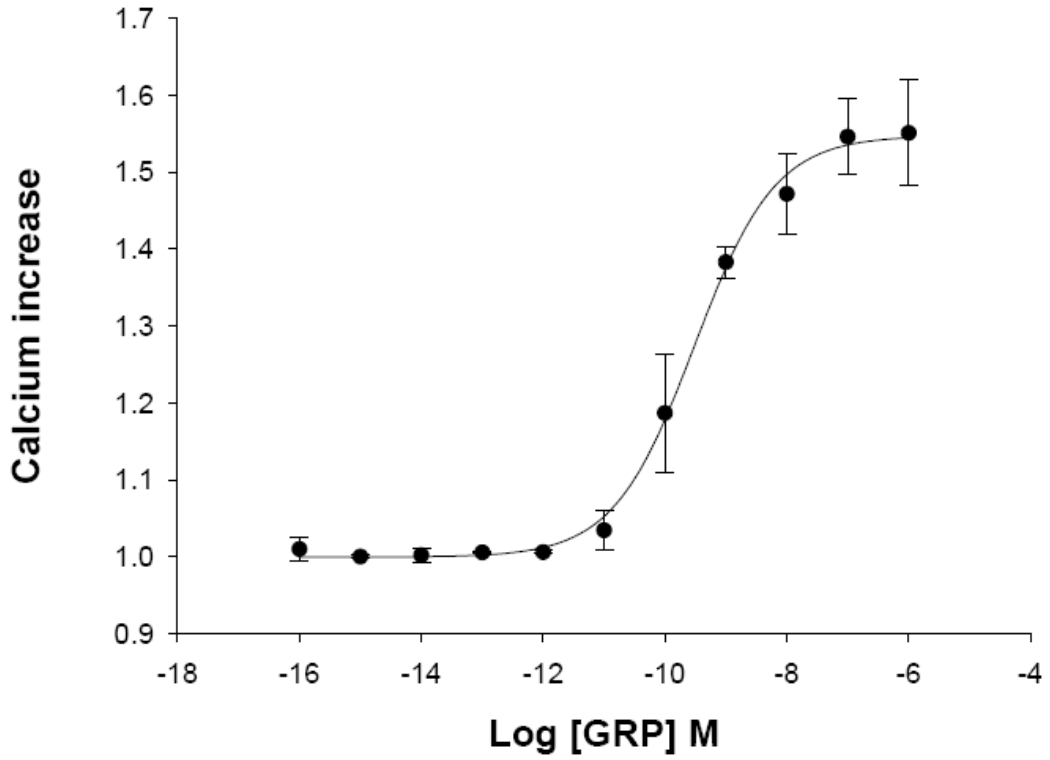
REF: P30150

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

-GASTRIN-RELEASING PEPTIDE RECEPTOR (GRPR) / (BB_2) CELL LINE -



Product name: GRPR (BB_2) /U2OS cell line

Ec_{50} GRP: 3.02 x 10^{-10} M

Z´: 0.71+/- 0.02

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REF: P30150

- GASTRIN RELEASING PEPTIDE RECEPTOR (GRPR) / (BB_2) U2OS CELL LINE -

Product Name:	GRPR (BB_2)/U2OS
Official Full Name:	Gastrin releasing peptide receptor
DNA Accession Number:	GenBank: NM_005314
Host Cell:	U2OS
Format:	2 cryopreserved vials
Resistance:	G418
Size:	> 3 x 10^6 cells / vial
Storage:	Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker GRPR contains U2OS cells stably expressing human Gastrin releasing peptide receptor (GRPR) with no tag.

Innoprot HiTSeeker GRPR cell line has been designed to assay compounds or analyze their capability to modulate Gastrin releasing peptide receptor. When the agonist binds to GRPR a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (Calcium).

This cell line has been validated measuring calcium increase in the cytosol. The high reproducibility of this assay allows monitoring GRPR activation process in High Throughput Screening.

About GRPR

The gastrin-releasing peptide receptor (GRPR), properly known as Bombesin Receptor 2 (BB_2) is a G protein-coupled receptor whose endogenous ligand is gastrin releasing peptide.

The **bombesin receptors** are a group of G-protein coupled receptors :

BB_1 or Neuromedin B receptor (NMBR)

BB_2,or Gastrin-releasing peptide receptor (GRPR)

BB_3, or Bombesin-like receptor 3 (BRS3)

The **gastrin-releasing peptide receptor** (**GRPR**), also known as **BB_2** is highly expressed in the pancreas and is also expressed in the stomach, adrenal cortex and brain. Gastrin releasing peptide receptor is thought to be implicated in several types of human cancer

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Assay Characterization

Our expression plasmid contains the coding sequence of human GRPR protein. Our plasmid was transfected in U2OS cells. Resistant clones were obtained by limit dilution and receptor gene expression was tested by RT-PCR using GAPDH as internal control (Fig.1).

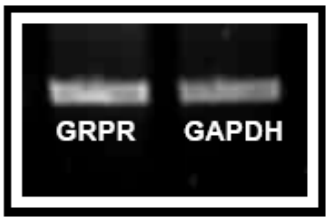

Fig.1. GRPR and **GAPDH housekeeping gene RT-PCR.**

Validation of GRPR cell line

Calcium assay (Ec50 = 3.02 x 10^{-10}M)

A typical fluorescent calcium assay was performed using Fura-2/AM ratiometric. Calcium increase inside the cell was measured using the ratio of the fluorescence from Fura2 bound and not bound to the ion. Image acquisition was performed using a "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Cells were incubated with Fura2-AM and treated with increasing GRP concentrations.

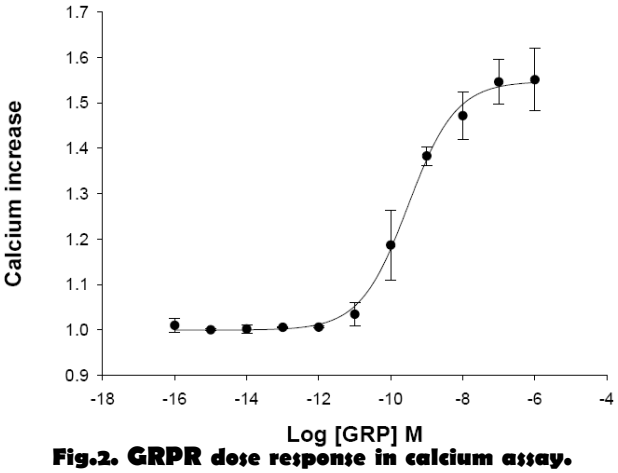

Fig.2. GRPR dose response in calcium assay. Cells were treated with **GRP** concentrations ranging from 0 to 1 µM, n=5. The EC50 for **GRPR** was ~**3.02x10^{-10}M**. The calcium assay was validated with a Z´= 0.71+/- 0.02 for High Content Screening.

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